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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Heyman et al.	Art Unit:	1646
Application No.:	09/937,112	Examiner:	C.B. Wilder
Filed:	May 13, 2002		
Title:	METHODS OF OBTAINING FULL-LENGTH NUCLEIC ACID SEQUENCES USING *E. Coli* TOPOISOMERASE III AND ITS HOMOLOGS		

Mail Stop Non-Fee Amendment
Commissioner for Patents,
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT IN RESPONSE TO OFFICE COMMUNICATION

Sir:

Responsive to the Restriction Requirement mailed May 20, 2003, consideration of the following remarks respectfully is requested.

CERTIFICATION UNDER 37 CFR §1.8
I hereby certify that the documents referred to as enclosed herein are being deposited with the United States Postal Service as first class mail on this date, June 19, 2003, in an envelope addressed to: Mail Stop Non-Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.
Cecilia Tobin *(Name of Person Mailing Paper)*
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I. AMENDMENT

Please amend claim 4 as indicated. Upon entry of the amendment, the status of the claims will be as follows:

1. (Original) A method of producing full-length coding sequences, said method comprising:

(a) synthesizing first strand cDNA using isolated full-length mRNA from a population of cells as a template, thereby forming first strand cDNA/mRNA hybrid(s);

(b) denaturing the first strand cDNA/mRNA hybrid(s);

(c) attaching a non-native tag sequence to the 3' end of the first strand cDNA; and

(d) producing a full-length double-stranded cDNA by synthesizing second strand cDNA using the tagged first strand cDNA produced in step (c).

2. (Original) A method according to claim 1 wherein the mRNA is isolated employing an affinity purification material.

3. (Original) A method according to claim 2 wherein the affinity purification material comprises one or more cap-binding proteins bound to a solid surface.

4. (Currently amended) A method according to claim 3 wherein the cap-binding protein(s) are selected from the group consisting of eIF4E, ~~eIF4G,~~ eIF4F, eIF4G, nCBP, and eIF4E:eIF4G fusion protein.

5. (Original) A method according to claim 2 wherein the mRNA to be isolated comprises a biotinylated cap structure.

6. (Original) A method according to claim 5 wherein the affinity purification material is a streptavidin or avidin-complexed solid support.

7. (Original) A method according to claim 1 wherein the mRNA is de-capped and de-phosphorylated after isolation.

8. (Original) A method according to claim 1 wherein the tag sequence comprises a recognition site for a site-specific recombinase.

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9. (Original) A method according to claim 8 wherein the tag sequence further comprises a recognition site for a site-specific restriction endonuclease.

10. (Original) A method according to claim 1 wherein the tag sequence is attached by a site-specific recombinase capable of recognizing and acting on single stranded DNA.

11. (Original) A method according to claim 10 wherein the site-specific recombinase is *E. coli* topoisomerase III.

12. (Original) A method according to claim 1 further comprising amplifying the cDNA during or after step (d).

13. (Original) A method according to claim 12 further comprising inserting the amplified cDNA into an expression vector.

14. (Original) A method according to claim 1 further comprising treating the first strand cDNA/mRNA hybrid(s) formed in step (a) with a substance that degrades single stranded RNA;

and isolating the undegraded hybrid(s) with an affinity purification material having affinity for capped mRNA prior to performing step (b).

15. (Original) A method according to claim 14 wherein the substance is RNAse I.

16. (Original) A method according to claim 14 wherein the affinity purification material comprises one or more cap-binding proteins bound to a solid support.

17. (Original) A method according to claim 14 wherein the mRNA component of the cDNA/mRNA hybrid comprises a biotinylated cap structure.

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18. (Original) A method according to claim 17 wherein the affinity purification material is a streptavidin or avidin-complexed solid support.

19. (Original) A method according to claim 14 further comprising inserting the double stranded cDNA resulting from step (d) into an expression vector.

20. (Original) An isolated full-length coding sequence prepared according to the method of claim 1.

21. (Original) An expression vector comprising an isolated full-length coding sequence prepared according to the method of claim 1.

22. (Original) An expression vector according to claim 21 comprising one or more elements selected from: a promoter-enhancer, a selection marker encoding sequence, an origin of replication, an epitope-tag encoding sequence or an affinity purification-tag encoding sequence.

23. (Original) An expression vector according to claim 22 wherein the promoter-enhancer is the T7 promoter, gal1 promoter, metallothionein promoter, AraC promoter, or CMV promoter-enhancer.

24. (Original) An expression vector according to claim 22 wherein the selection marker encoding sequence encodes a protein which imparts antibiotic resistance to cells.

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25. (Original) An expression vector according to claim 22 wherein the epitope-tag sequence encodes V5, the peptide Phe-His-His-Thr-Thr (SEQ ID NO:2), hemaglutinin, or glutathione-S-transferase.

26. (Original) An expression vector according to claim 22 wherein the affinity purification-tag sequence encodes a polyamino acid tag or a polypeptide.

27. (Original) An expression vector according to claim 26 wherein said polyamino acid tag is polyhistidine.

28. (Original) An expression vector according to claim 26 wherein said polypeptide is a chitin binding domain or glutathione-S-transferase.

29. (Original) An expression vector according to claim 26 wherein said polypeptide encoding sequence includes an intein encoding sequence.

30. (Original) An expression vector according to claim 21 wherein the expression vector is a eukaryotic expression vector or a prokaryotic expression vector.

31. (Original) An expression vector according to claim 30 wherein the eukaryotic expression vector is pYES2, pMT, pIND, or pcDNA3.1.

32. (Original) A method of obtaining full-length coding sequences comprising:

(a) contacting full-length mRNA, isolated from a population of cells by employing an affinity purification material, with reverse transcriptase and thereby synthesizing first strand cDNA and forming first strand cDNA/mRNA hybrids;

(b) treating the first strand cDNA/mRNA hybrids with a substance that degrades single stranded RNA;

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(c) isolating undegraded hybrid(s) from degraded hybrids employing an affinity purification material having affinity for capped mRNA;

(d) denaturing the isolated cDNA/mRNA hybrids obtained from step (c) thereby producing single stranded cDNA and single stranded mRNA;

(e) attaching a non-native tag sequence to the single-stranded cDNA, wherein the tag sequence comprises a site-specific recombination sequence and is attached by *E. coli* topoisomerase III; and

(f) synthesizing second strand cDNA using the tagged cDNA as a template and/or amplifying the cDNA, wherein the amplification primers comprise an anti-coding sequence of the tag sequence (5') and oligo-dT (3').

33. (Original) A method according to claim 32 further comprising inserting the cDNA obtained in step (f) into and expression vector.

34. (Original) A fusion protein comprising eIF4E and eIF4G.